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(54) Title: DEVICE AND METHOD FOR MIXING MAGNETIC PARTICLES WITH A FLUID

(57) Abstract: This invention relates to the use of magnetic or magnetizable particles, and, in particular, to methods of mixing magnetic or (super)paramagnetic particles efficiently with a fluid and the separation of the magnetic particles from a fluid, optionally followed by resuspension of the particles in another fluid. The present invention provides a method of mixing, in one or more container(s), magnetic or (super)paramagnetic particles with a fluid, using more than one magnets, whereby the containers are subjected to magnetic fields with different and changing directions by moving the magnets with respect to the position of the container(s) and/or by moving the containers with respect to the positions of the magnets. The invention further provided a device for doing the same. Preferably the holders for the containers and the magnets in the device are placed in intervening array geometries and the magnets are placed in line in such a way that all magnets that are in line have their poles oriented in the same direction, and that all magnets in a neighboring line have their poles oriented in the reverse direction with respect to the poles of the magnets in the first line.

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Device and method for mixing magnetic particals with a fluid

This invention relates to the use of magnetic or magnetizable particles, and, in particular, to methods of mixing magnetic or (super) paramagnetic particles efficiently with a fluid and the separation of the magnetic particles from a fluid, optionally followed by resuspension of the particles in another fluid.
The invention further provided a device for doing the same.

Magnetic particles are often used in separation processes. There are many biological assay methods and purification methods in which magnetic particles are used. For example, immuno assay methods, nucleic acid hybridization assays and the like. Magnetic particles can also be used in purification methods, to isolate particular components, proteins, nucleic acids, from the material in which they were contained. The particles can be used to separate certain components from a mixture, for example, because they are coated with a reagent with a specific affinity for the component. Magnetic particles can be drawn to, for example, the wall of a container in which the fluid with the magnetic particles was contained and the fluid can be removed and, optionally, be replaced with another fluid. Thus, the particles can be mixed with the fluid from which the specific component is to be removed, the component will bind to the magnetic particle, and a magnet can be used to separate the particles with the component from the remainder of the mixture in the fluid. Optionally the magnetic particles can be washed, and can be separated in another fluid. Or the component can be removed from the particles again into another fluid.

The use of magnetic particles for purifying a nucleic acid (NA) target from a biological sample is well known.
Purification methods for nucleic acid using magnetic particles have for example been described in EP757106 (Toyobo) and WO 96/41811 (Boehringer Mannheim). In these applications methods are described wherein a sample solution containing nucleic acid is treated with a chaotropic substance to release the nucleic acid. After releasing the NA from the biological entity in the lysis buffer, the NA is bound to the magnetic particles. Both particles coated with a target-specific probe as well as particles having a metal oxide coating (e.g. silica), giving a generic binding of all NA contained in the sample are used for this purpose. After binding the target, interfering components such as cell debris, enzymes, proteins anti-coagulants and salt are removed by washing the magnetic particles in a (set of) wash buffer(s). Finally, the purified NA is released from the particles by mixing the particles in a small volume of elution buffer. This process is called elution since it is the nucleic acid that is eluted from the particles.

For efficient washing and elution the magnetic particles need to be well dispersed and mixed in the relevant buffers. In general, this washing and elution process may be

hampered by the aggregation or clogging of the magnetic particles either caused by the adsorption on the magnetic particles of specific components in the lysed sample (e.g. genomic DNA) or by residual magnetic dipole fields induced in the particles. In particular, the use of silica coated (magnetic) particles with samples that contain significant amounts of genomic DNA (whole blood, sputum, tissue), results in a tight pellet that is difficult to process.
Well-known methods for mixing (magnetic) beads in a liquid buffer are vortexing, sonification or pipetting. These methods however are difficult to automate, and/or give risk of sample to sample contamination by aerosol generation or they may degrade the NA target. Furthermore, these methods are not well suited for very small volumes of liquid (typically 0.01ml) as may be required for the elution process.

The method and device according to the invention are especially suitable for use with isolation procedures, where, usually an ingredient is to be isolated in rather pure form from a relatively large volume of sample fluid, and concentrated into a smaller volume of another fluid to be suitable for further use.
In the case of a method for the isolation of nucleic acid such further use may be a nucleic acid amplification method or an assay for the detection of nucleic acid or both.

A method and apparatus for separating and resuspending superparamagnetic particles is disclosed in WO 91/09308 (Diatec instruments).
In this application it was disclosed that superparamagnetic particles may be aggregated and resuspended by subsequent application of different magnetic fields. First and second applications of the magnetic field could be provided with the same magnet, which was then rotated around the container containing the particles to a different location. Two spaced opposed electromagnets, however, could also be used. These electromagnets were energized alternately to produce the first and second magnetic fields that keep the particles in suspension and mix them with the fluid in which they were contained.
A method for the separation of magnetic particles from a fluid is disclosed in US 3985649. The particles may be separated from a fluid by bringing the particles into close proximity with a magnet and moved trough the liquid along the wall of a container. They may even be moved out of the liquid in this way and can be transported to a second container.
In US4988618 a device is described for use with assays wherein multiple small volume samples are tested at the same time. These type of assay can be performed in, for example, microtiter plates. Magnetic microparticles are present in each well of the microtiter plate. The device thus has multiple orifices and the orifices are each surrounded by multiple permanent magnets, preferably four. The resulting structure of magnets and orifices is rigid; the magnets are not intended to be moved and are mounted in fixed relations with respect to themselves and to the base of the device. All magnets are aligned and the field orientation of the magnets may b such that all magnets have the same field direction or neighboring magnets have opposite field directions. The magnet orientation thus r sults in four spot attraction sites per orifice. The magnets are purely

meant for separation purposes. It is disclosed in the patent that the device may further comprise means or agitating the reagents within the containers.

The present invention relates to a method and device, which allows efficient mixing of magnetic or magnetizable particles in a fluid, and optionally separation of the particles from said fluid. Use is made of magnetic field of opposite and changing directions. It has been found that, when magnetic or magnetizable particles in a fluid are subjected to these magnetic fields, the particles are, under the influence of the field, efficiently contacted with the fluid. Such particles normally may tend to form a clot, which can prevent efficient mixing with a fluid. It has been found that, by subjecting the container in which the fluid and the particles are comprised, to magnetic fields of different and changing directions, the particles are efficiently separated from each other and drawn trough the fluid in such a way that a very efficient mixing process occurs. The method allows efficient mixing of particles with even very small fluid volumes. The method of the invention therefore has the advantage that it may save in, for example, washing fluids and may allow the reduction of the volume of fluid needed. Thus, for example in isolation procedures, the method of the invention allows the purification of reagents in high concentrations. Beside, whereas prior art methods can be laborious and time consuming, the method is fast and easy to perform.

Thus, provided with the invention is a method of mixing, in one or more container(s), magnetic or (super)paramagnetic particles with a fluid, using more than one magnets, whereby the containers are subjected to magnetic fields with different and changing directions by moving the magnets with respect to the position of the container(s) and/or by moving the containers with respect to the positions of the magnets.

With "mixing" in this context is meant that the particles and the fluid are brought in close contact. Mixing thus, means "contacting" in a very efficient manner, such as when particles would be washed or reacted with components present in the fluid. Mixing, in this context, does not necessarily provide a homogeneous mixture after the process is finished. The particles may, when the magnets are removed, segregate to the bottom of the container in which they are comprised or may be held to the wall of the container in a particular location by the magnets. The mixing process can for example be used to wash the particles or to react the particles with a component of the liquid, or to bind a component of the liquid to a reagent coated on the particles. Likewise, the mixing process may result in the elution of a certain component originally present on the particles into the surrounding liquid. The method of the invention is applicable in each of these processes and provides an efficient rapid and convenient way of contacting magnetic or magnetizable particles with a volume of a certain fluid.

The present invention thus provides a generic method for mixing magnetic particles with a fluid almost indep ndent of their lev l of pelleting/aggregation. The method further allows releasing of reagents bound to the particles, for example nucleic acid, from the particles and concentration into a small volume. The method is easy to automate and well suited for high throughput formats. It minimizes the risk of contamination by droplets or aerosols.

During a washing (or elution) cycle the (aggregated) particles are dragg d through the liquid from left to right by placing a first magnet close to the outside right wall of the vessel and subsequently withdraw this first magnet and simultaneously place a second magnet close to the opposite (left) wall of the vessel in order to drag the particles into the opposite direction. The present invention furthermore provides a device for performing said method.

The device according to the invention comprises means for holding the containers and more than one magnets and means for moving said magnets with respect to the position of said containers and/or means for moving said containers with respect to the position of said magnets in such a way that the containers are subjected to magnetic fields with different and changing directions.

Preferably the magnets are moved with respect to the containers.

The containers may have any convenient shape. Any vessel, suitable for holding a fluid sample in which magnetic particles are dispersed can be used. Preferably the vessels are suitable for holding small liquid samples. For example, they may be Eppendorf cups, PCR containers or micro-titer plate strips).

The magnets may be placed in different geometries with respect to the containers. Any geometry which allows the movement of the magnets with respect to the position of the containers or the other way around, and which will result in magnetic fields of different and changing polarity in each container can be used.

It was found that this washing (or elution) process become particular efficient with the two magnets arranged in such a way that they strongly repel each other (by facing each other with similar poles N-N or S-S). Due to this arrangement the magnetic field lines in the area in the vessel where the magnetic beads are located show a strong and sudden change in direction during each cycle. When the container is placed between two magnets that strongly repel each other because their similar poles are facing each other (N-N or S-S) the slightest movement of either one of the magnets or of the container with respect to each other will result in sudden strong changes of the magnetic field to which the particles in the container are subjected. It has been found that this results in a very efficient way of mixing the particles with the fluid, even when the particles as such tend to form a clot or had already formed a clot within the fluid.

The magnets are preferably arranged in such a way that each magnet repels each of its neighboring magnets.

The magnets may be placed in line in such a way that magnets of opposite polarities can be moved back and forth on straight parallel paths along opposite sites of each container in such a way that the direction of the magnetic field in each container is repeatedly reversed.

This may advantageously be achieved by placing the magnets in line in such a way that all magnets that are in line have their poles oriented in the same direction, and that all magnets in a neighboring line, that is on th other side of the containers next to the first line of magnets, have their poles oriented in the reverse direction with respect to the poles of the magnets in the first line.

When the magnets are moved, this may result in the containers being repeatedly placed between two magnets that face each other with the same pole.
The magnets and containers may be placed in parallel rows and the rows of magnets can be moved in opposite directions alongside the rows of containers.
But, of course, based on the basic concept of the method of the invention other geometries can likewise be devised.
The basic concept of an embodiment of a device according to the invention wherein the magnets are movable with respect to the containers is illustrated in Fig.1. The magnetic particles are in a liquid buffer contained in a vessel. The (aggregated) particles are dragged through the liquid from left to right and v.v. by translating a set of at least two magnets arranged such that the magnetic field induced in the vessel changes polarity upon each movement of the magnets.
The method can be used with more containers and magnets. Thus the method and device according to the invention allow for batch-wise processing of several vessels simultaneously. The method and device according to the invention are especially suitabl for treating a large number of fluid volumes in each of their respective containers at the same time.
In a preferred embodiment of the device according to the invention the containers and the magnets are placed in intervening array geometries. This layout allows the use of the method of the invention to give a high throughput format.
An embodiment wherein the containers and the magnets are placed in intervening array geometries is illustrated in fig.2. The vessels (e.g. Eppendorf cups, PCR containers or micro-titer plate strips) are placed in an array geometry with the magnets fixed to a second array that translates with respect to the vessels.
In this way a large series of samples is processed simultaneously. Addition and aspiration of liquids may be by hand or by an automated multi-tip dispenser instrument as know in the art.

The method of the invention may also be used with a closed system. That is, a system wherein the liquid, for example, is not contained in a vessel, but in a tube. Thus, with containers, as used with the method of the invention, not only containers used in batch wise processes are meant but also containers used in closed systems, such as tubes and the like. Such an alternative embodiment of a device according to the invention illustrated in figure 3. The particles and liquid are not contained in a vessel but in a tube, allowing processing the particles in a closed system.

Depending on the exact intended use of a device of the present invention several modifications and variations on the above-described theme are possible. For example, the shape of the container may be modified and further modifications as to the location of the magnets with respect to said containers can be made as well.
A device according to the present invention is especially suitable for use with methods for the purification of, for exampl , nucleic acid from biological starting material.
For a specific purpose the device can be further modified to match the intended use.

The adjustments may result in better ways for separating the particles from the liquid. The device may also be adjusted in such a way that it can be used with different sample fluid volumes.

In a preferred embodiment according to the invention the magnets can not only be moved with respect to the position of the containers but can also be moved in a direction along the walls of the containers (which would be vertical, when the containers are in an upright position).
In this way, the position of the magnets can be adjusted according to the volume of the fluid in the containers. Thus, when there is only a very small fluid volume to be mixed with the particles the magnet will be in a position that is lower than the position it will have when there is a larger volume of fluid in the same container.

The fact that the magnets can be moved in a vertical direction has the additional advantage that the magnets can now also be used to draw the particles to the lower part of the container, even when a bigger fluid volume is used. Thus, this allows the removal of a large part of the fluid volume, for example by a pipettor, while the magnet holds down the particles.
Optionally, the magnets, when they can be moved in a vertical direction along the walls of the containers, can also be used to draw the particles alongside the wall of the container till a position above the surface of the fluid. In that way the particles can be separated from the fluid and the remaining fluid may be removed from the container or, for example, be replaced by another fluid after which the particles may be drawn down below the liquid level and mixed with the new fluid using the magnets.
It is evident that the design of the device allows many variations in the methods of its use and all fall within the scope of the invention.
The use of the movement of the magnets in a vertical direction is illustrated in figure 4.

To allow the use of the device with a procedure involving the subsequent treatment of the particles with several liquids in different volumes and achieve an efficient mixing and separation of the particles with/from the respective fluids, adjustments can be made to the containers as well.
If a large container is used with a very small fluid volume the problem may arise that the particles can no longer be contacted with the fluid, simply because the fluid volume is more or less spread out over the bottom of the container and doesn't even cover the particles.
Thus, containers can be devised that can be used with different liquid volumes and still allow efficient mixing of the fluid volumes with the particles. Such containers and the use thereof are likewise part of the present invention.
To allow the use of fluids of considerable different volume a container can be used that comprises a part that is suitable for containing small fluid samples, while this part is connected to a part that is suitable for containing large volume samples. An example of such a container is illustrated in figure 4.

The multi-purpose container as depicted in figure 4 is provided with a tip with a relatively small diameter suitable for containing small volume samples, while the part on top of the tip is suitable for containing larger volume samples.
As indicated in figure 4 this container is suitable for using the device with small and large fluid volumes and the height of the magnets with respect to the container can be adjusted accordingly.
Moreover, the tip allows the collection of the particles from a large volume sample by moving the magnets in the downward orientation. The major part of the liquid can then b removed from the container without accidentally removing any of the particles.

A device according to the invention is especially suitable for use in a method for the isolation of nucleic acid from biological samples.
A typical method for the isolation of nucleic acid is the method as devised by R.Boom et al., as disclosed in EP 389063.
The "Boom method" involves the treatment of the biological material with a lysis buffer containing a chaotropic substance such as guanidine-isothiocyanate and a siliceous solid phase. The siliceous solid phase may be provided in the form of magnetic silica particles. The nucleic acid released from the material by the lysis buffer will adhere to the (magnetic) siliceous particles. Thus, the particles and the biological material in the lysis buffer should be thoroughly contacted with each other, which is where the use of a device according to the method would come in. The particles with the nucleic acid adhered thereto can subsequently be separated from the remainder of the sample using a magnet (which can also be done with a device according to the invention provided that it is adapted for that purpose). Subsequently the nucleic acid containing particles should be washed, which requires the mixing of the particles with a washing buffer. This is another function that may be performed by the device according to the invention. The particles are then removed from the washing liquid and contacted with an elution buffer (again, thorough contact between the particles and the elution buffer is required) and the nucleic acid is thus released from the particles into the elution buffer. In general, liquid volumes required for washing will be about 10 times larger than for elution. A typical volume for washing (per vessel per wash step) is 0.2-0.5 ml. The typical volume for elution buffer is 0.010/0.050ml

The embodiment of the device wherein the magnets can be moved in the vertical direction as well and containers are used that have a tip for the use of smaller liquid volumes is especially suitable for use with the so-called "Boom method" for the isolation of nucleic acid as described above.
When the device would be used with a method like the Boom method this can be performed with the following procedur :
A typical volume required for a washing step would be 0.2 to 0.5 ml, which is a relatively large volume. Therefore, during washing the magnetic particles are in the upper part of the vessel (level 1, fig.4 situation 1). However, for most applications the nucleic acid target needs to be concentrated in a buffer volume of typically 10 to 50µl. Such small

liquid volumes are hard to handle. It is difficult to control the size of such a small volume as well as to manipulate it in a vessel in combination with magnetic particles to form a suspension for performing bound-free steps.

Fig.4 shows a method that overcomes the above difficulties.

After completing the washing procedure the particles are captured at the side of the vessel wall (level 1, situation 1) and the wash liquid is aspirated with a pipetter tip.

Next, the vessel is filled with fresh elution buffer (about 0.2ml) and the magnetic particles are transported down to the lower end of the vessel (level 3) by bringing the magnets down (situation 2). Transport of particles can be accelerated by translating the magnet array as is done during washing as it moves downward. The composition of the ET buff r is such that no nucleic acid is released from the silica as long as the buffer temperature is not above RT.

Next, while aspirating, the tip is introduced into the vessel until its lower end is at a level that corresponds to the required volume of ET buffer (e.g. 10µl, see situation 3).

Next, a heat block is brought into contact with the vessel to heat up the temperature of the buffer to 55-60°C (situation 4)

Next, the actual elution procedure starts by translating the magnets horizontally as during the washing procedure, but now at level 3. Preferably, during elution, the heat block remains in contact with the vessel to keep the temperature of the elution buffer at 55-60°C.

Finally, after completing the elution, the heat block is moved away from the containers (down)l and the magnets are moved up to level 2 (situation 5) to withdraw the particles from the elution buffer that is now ready for further processing (amplification, sequencing,)

Preferably, in order to allow the heat block to contact the vessel during elution without disturbing the elution process (situation 4), the heat block has a special design that accounts for the dimensions of the magnetic array as well as for the shape of the vessel. The heat block preferably is produced from a material that is non-magnetic. For example, the heat block is produced from aluminum and contains a ceramic heater element as is known from the state of the art.

Thus, it is illustrated how the device can be used to automate and speed up existing procedures, that now have to be perform, either by hand or in more complicated automated devices.

Of course, the use of a device according to the invention will find its application in many biological assays or purification processes.

BRIEF DESCRIPTION OF THE FIGURES:

Figure 1: The basic concept of an array according to the invention

Figure 2: Device wherein the holders for the containers and the magnets are placed in intervening array geometries and the magnets are placed in line in such a way that magnets of opposite polarities can be moved back and forth on straight

parallel paths along opposite sites of each container in such a way that the direction of the magnetic field in each container is repeatedly reversed.

Figure 3: Device wherein the containers are part of a closed syst m, e.g. a tube.

Figure 4: Device wherein the magnets can also be moved in a vertical direction so as to be positioned at different heights with respect to the walls of the containers and the containers are tube-shaped vessels provided with a tip for holding small liquid volumes.

Claims:

1. Method of mixing, in one or more container(s), magnetic or (super)paramagnetic particles with a fluid, using more than one magnets, whereby the containers are subjected to magnetic fields with different and changing directions by moving the magnets with respect to the position of the container(s) and/or by moving the containers with respect to the positions of the magnets, characterized in that the magnets and the holders for the containers are placed in intervening array geometries.

2. Method according to claim 1, wherein the containers, by moving either the containers or the magnets, are subjected to magnetic fields of opposite polarity.

3. Method according to claim 1 or 2, wherein, as a result of moving either the magnets or the containers, the containers are repeatedly moved between two magnets that face each other with the same pole.

4. Method according to any of claims 1-3, wherein the magnets are moved with respect to the position of the containers or the containers are moved with respect to the position of the magnets in such a way that the magnetic or (super)paramagnetic particles are moved trough the fluid to one side of the container by bringing a first magnet with its magnetic pole close to the wall of the container and, subsequently are moved to the opposite side by bringing a second magnet close to the opposite wall of the container, whereby said second magnet has the same magnetic pole as the first magnet..

5. Method according to any of the preceding claims, wherein the magnets are moved with respect to the containers.

6. Device for mixing magnetic or (super)paramagnetic particles in one or more containers with a fluid, said device comprising means for holding said one or more containers and more than one magnets and means for moving said magnets with respect to the position of said containers and/or means for moving said containers with respect to the position of said magnets in such a way that the containers are subjected to magnetic fields with different and changing directions.

7. Device according to any of claims 1-6, the device being provided with a heat block that is positioned in such a way that it can be moved into close proximity with the containers so as to warm their contents, and moved away again.

8. Device according to claim 7, wherein the heatblock is positioned underneath the containers and has wells which enclose the tips of the containers when the heatblock is brought into close proximity with the containers.

9. Device according to claim 1 wherein each magnet is oriented in such a way that it repels each of its neighboring magnets.

10. Device according to any of claims 1-9, wherein magnets can be moved back and forth on straight parallel paths along opposite sites of each container in such a way that the direction of the magnetic field in each container is repeatedly reversed.

11. Device according to claim 1, wherein the magnets are placed in line in such a way that all magnets that are in line have their poles oriented in the same direction, and that all magnets in a neighboring line have their poles oriented in the reverse direction with respect to the poles of the magnets in the first line.

12. Device according to any of claims 1-11, wherein the magnets can also be moved in a vertical direction so as to be positioned at different heights with respect to the walls of the containers.

13. Device according to any of claims 1-12 wherein the containers are part of a closed system.

14. Device according to any of claims 1-13 wherein the containers are tube-shaped vessels provided with a tip with a smaller diameter.

15. Use of a device of any of claim 6-13 in a method for the isolation of nucleic acid.

16. Method for the isolation of nucleic acid from starting material comprising the following steps:

(a) bringing the starting material together with an appropriate lysis buffer and magnetisable silica particles into one or more containers of a device according to claim 11,

(b) mixing the ingredient of the vessels by moving the magnet array with respect to th containers in such a way that the direction of the magnetic field in each container is repeatedly reversed for a sufficient amount of time with the magnets at a height that is adjusted to the volume of the sample,

(c) collecting the particles at a wall of the container using the magnets,

(d) removing most of the sample liquid from the device,

(e) adding a sufficient amount of washing buffer to the device,

(f) repeating step (b) to (d),

(g) adding a suitable amount of elution buffer to the device,

(h) drawing the particles down into the tip of the container by moving the magnets to a lower position

(i) Optionally heating the container by moving a heatblock into close proximity with the containers.

(j) optionally removing an appropriate amount of elution buffer from the device

(k) repeat step (b),

(l) move the magnets in a vertical direction to a position above the fluid level,

(m) collect the elution buffer with the isolated nucleic acid container therein.

Fig.1

Side view

Top view

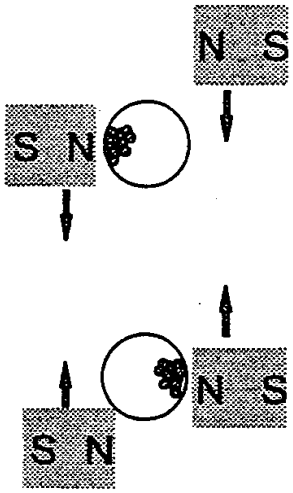


Fig.2

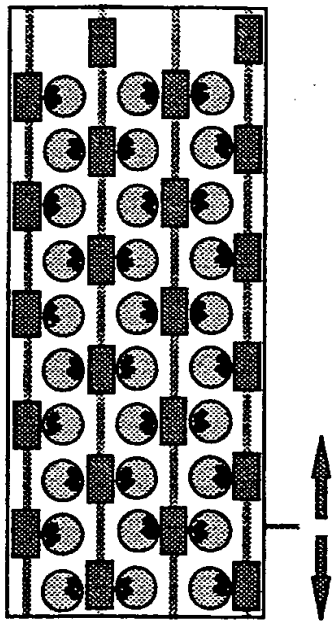


Fig.3

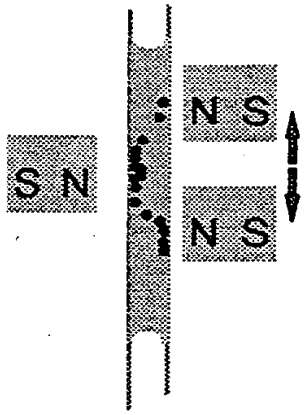
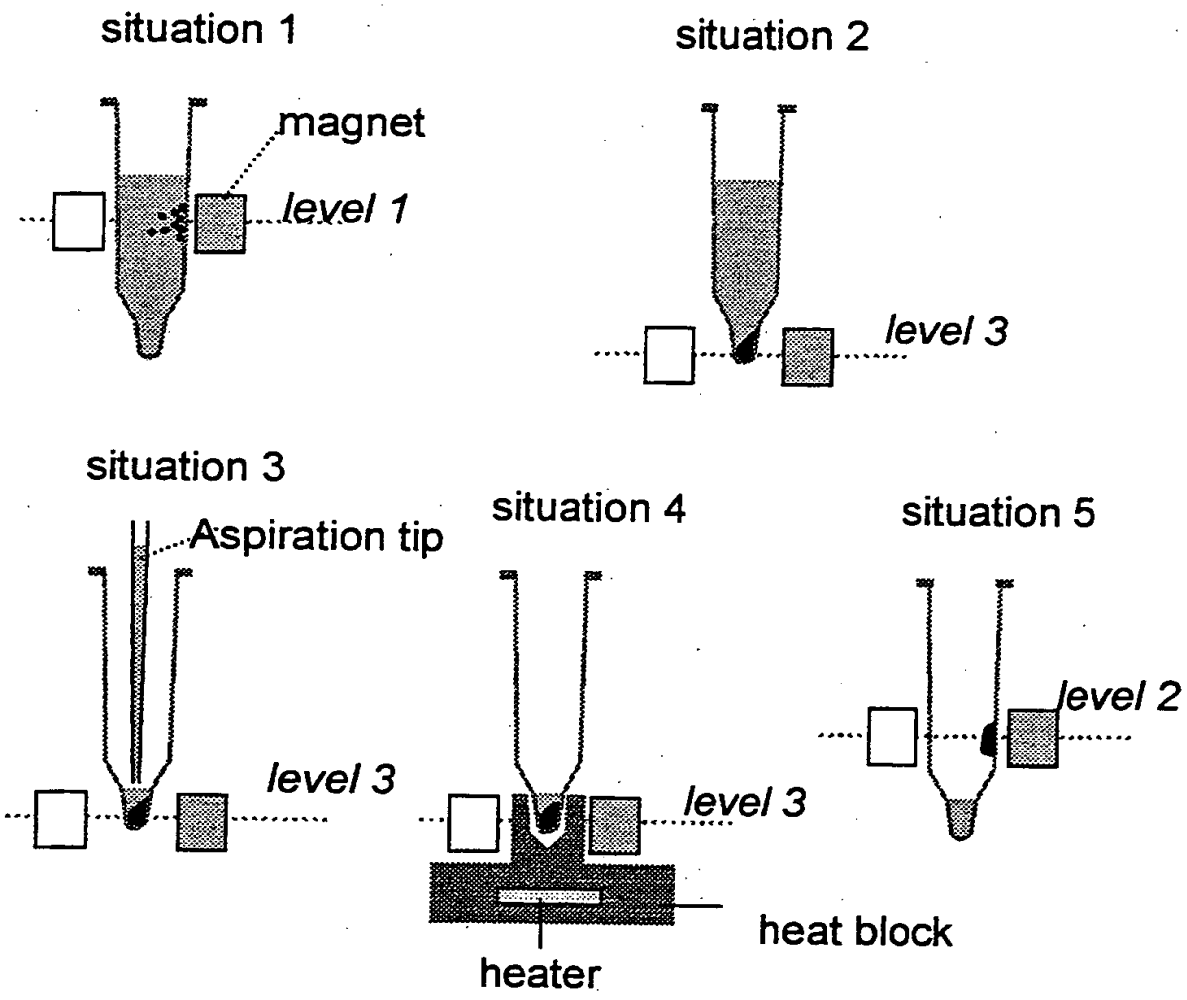


Fig.4


situation 1
magnet
level 1
situation 2
level 3
situation 3
Aspiration tip
level 3
situation 4
level 3
heat block
heater
situation 5
level 2

PATENT COOPERATION TREAT

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Applicant KREUWEL, Hermanus, Johannes, Maria et al	

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13 February 2001 (13.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

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(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
99480 WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06789	14/07/2000	19/07/1999
International Patent Classification (IPC) or national classification and IPC B03C1/28		
Applicant AKZO NOBEL N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/02/2001	24.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Herry, M Telephone No. +49 89 2399 8666

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/06789

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-9 as originally filed

Claims, No.:

1-16 as originally filed

Drawings, sheets:

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5, 7-8, 10, 12-13, 16
	No:	Claims	1-4, 6, 9, 11, 14-15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	5, 7-8, 10, 12-13, 16
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

ITEM V:

See also Item VIII of the present communication.

Documents US-A-5 770 461, EP-A-0 691 541 and US-A-5 558 839 were not cited in the international search report.

Concerning independent claim 1:

The subject matter of claim 1 is not considered to be new (Article 33(2) PCT).

US-A-5 770 461 (D1: fig.6) discloses a method of mixing, in several containers (1), magnetic particles with a fluid (c.1, l.6-10), using more than one magnets (27a-27h), whereby the containers are subjected to magnetic fields with different and changing directions by moving the containers with respect to the positions of the magnets (c.9, l.3-7). The magnets and the holders for the containers are placed in "intervening" (see ITEM VIII) array geometries (fig.6; c.9, l.8-16).

The applicant's attention is drawn to the fact, that these features are also known from WO-A-96 26011 (D2: figs.3-4; p.2, l.13-22; p.9-10).

Concerning independent claims 6 and 15:

The features of claim 6 are the same as the features of claim 1, with the exception that the relative arrangement of the magnets and containers is not specified. For the reasons mentioned above, the subject matter of claim 6 is not considered to be new (Article 33(2) PCT).
In the same way, the subject-matter of claim 15 is not new (see D2: p.1, l.7-12).

Concerning independent claim 16:

The subject matter of claim 16 is not considered to involve an inventive step (Article 33(3) PCT).

Claim 16 differs over D1 through features h, i and l. Features (h) and (l) facilitate the introduction and removal of fluid medium, while feature (i) ensures a suitable temperature. These features are thus part of a juxtaposition, their inventive step can be assessed separately (PCT Guidelines, Section IV, 8.3a). However:
- the step of heating the containers is obvious, since the necessity for a stable elevated temperature is well known (D1: c.4, l.6-11 in combination with US-A- 5558839, D3: c.3, l.4-8; c.11, l.6 and 37).
- moving vertically the magnets to a position opposite the "removal end" of the container, in order to facilitate manipulation of the fluid, is known from EP-A-0 691 541 (D4: figs.; c.5, l.38-45; c.6, l.37-42).

Concerning dependent claims 2-5 and 7-14:

These dependent claims do not seem to contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, since they are either known from the documents cited in the search report, or they come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen. In particular:

- The subject-matter of claims 2-4, 9, 11 and 14 is known from D1 (fig.6; c.9, l.51- 62). The features of claims 2-5, 9 are known from D2 (figs.3-4; p.9, l.16-21), as well as the features of claims 13 (p.22, l.1: an aseptic process) and 14 (figs.6-7).
- The provision of a heat block (claims 7-8) is obvious, since the necessity for a stable elevated temperature during incubation in the

container is well known (D1: c.4, l.6-11 in combination with US-A-5558839, D3: c.3, l.4-8; c.11, l.6 and 37).
- Moving the magnets relative to the containers (claim 10) is an obvious alternative to D1.
- Vertically movable magnets (claim 12) are known from EP-A-0 691 541 (D4: fig.4d; c.1, l.1-19; c.5, l.38-45; c.6, l.37-42) to facilitate the repeated removal and introduction of liquid medium.

ITEM VII:

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 to D4 is not mentioned in the description, nor are these documents identified therein.
The independent claims should have been correctly delimited in the two-part form according to Rule 6.3(b) PCT, and the features of the claims should have been provided with reference signs in parentheses (Rule 6.2(b) PCT).

ITEM VIII:

The claims are not clear (article 6 PCT) for the following reasons:

A) Although claims 1 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought and in respect of the terminology used for the features of that subject- matter. The aforementioned claims therefore lack conciseness.

B) The magnets and containers are essential to allow the mixing. However, the device of claim 6 comprises only "means for holding / moving" these magnets and containers. Thus claim 1 does not meet the requirement

following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

C) The feature "intervening array geometries" in claim 1 is not clear. The meaning of this feature is also rendered unclear by some statements made in the description (p.4, l.17-20; p.5, l.5-6 and 39-40).
Moreover no such array geometries can be seen in the embodiment considering one container and two magnets on opposite sides of the container.

Claim 4 (l.23) should have been clarified, since two magnets necessarily have the same magnetic poles.

D) The description contradicts the claims (PCT Guidelines, III-4.3):
Figures 1, 3, 4 and p.5, l.18-22, p.5, l.39-40 and l.43 (no "intervening array geometries") contradict claim 1. In the same way, page 4 (l.17-20) and p.5 (l.5-6) are in contradiction with claim 1.
The description p.6, l.37-39 indicates that a protection should be accorded to the containers themselves: such a statement is in contradiction with the claims.

PATENT COOPERATION TREATY

26 OKT. 2001

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

HERMANS Franciscus G.M.
P.O. Box 20
NL-5340 BH Oss
PAYS-BAS

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	24.10.2001
Applicant's or agent's file reference 99480 WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06789	14/07/2000	19/07/1999

Applicant
AKZO NOBEL N.V. et al.

19 07 2001
19 01 2002

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Salaün, M Tel.+49 89 2399-2126

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
99480 WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 06789	14/07/2000	19/07/1999
Applicant		
AKZO NOBEL N.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   Device and method for mixing magnetic particals with a fluid

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/06789

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B03C1/28 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B03C G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 26011 A (SIDDIQI IQBAL W) 29 August 1996 (1996-08-29) page 1, line 7 - line 12 page 2, line 13 - line 22 page 6, line 1 - line 17 page 14, line 18 - line 21 page 17, line 4 -page 18, line 26; claims 1,7,8,10,12-14,21; figures 3,4,7	1-6,8

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2000	07/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Decanniere, L

Form PCT/ISA/210 (second sheet) (July 1992)

INTER IONAL SEARCH REPORT

information on patent family members

Inte ional Application No
PCT/EP 00/06789

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9626011 A	29-08-1996	AT 172890 T	15-11-1998
		AU 4927496 A	11-09-1996
		DE 69600924 D	10-12-1998
		DE 69600924 T	10-06-1999
		EP 0810905 A	10-12-1997
		JP 11500952 T	26-01-1999
		US 6033574 A	07-03-2000

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